APPARATUS AND METHOD FOR INVESTIGATING CHEMICAL ENTITIES

Statement f Related Cases

[0001] This case claims priority of U.S. provisional patent application 60/443,824, which was filed on January 30, 2004, which is incorporated by reference herein.

Field of the Invention

[0002] The present invention relates to an apparatus and method for determining the structural parameters of unknown chemical entities through binding interactions.

Background

[0003] The structure of a complex chemical entity (*e.g.*, proteins) can be decoded via its binding activity with known chemical entities (*e.g.*, small molecules). But this requires the investigation of a very large number of such interactions. Current technologies (*e.g.*, fluorescent assays, *etc.*) and equipment for decoding the structure of proteins through binding require complex equipment and extensive development of assays as well as the selection of proper fluorescent labels. Other techniques, which decode structures directly (*i.e.*, without binding) are very complex (*e.g.*, mass spectrometers, *etc.*) and denature proteins in the process. As a consequence, there is a need for a relatively simple, low-cost, and high-throughput apparatus that is capable of monitoring binding interactions and obtaining the data required for identifying unknown chemical entities.

Summary

[0004] Some embodiments of the present invention are capable of monitoring binding interactions and obtaining data concerning the interactions. In accordance with the illustrative embodiment, binding activity is investigation directly, through its thermal signature. As a consequence, the illustrative embodiment of the present invention is a "label-free" technology, in that it does not require special assay development and labeling.

[0005] In accordance with the illustrative embodiment, an apparatus for monitoring and obtaining data on binding interactions includes an IR sensor, a sliding separator, and IR-transmitting fibers that are optically coupled, at a first end thereof, to the sensor. The sliding separator adjusts the spacing between fibers as is required for

interfacing the second end of the fibers with any of a variety of sample carriers. In some embodiments, the second end of the fibers is physically adapted to capture and immobilize chemical entities that are contained in the sample carriers.

[0006] After the chemical entities are engaged to the second end of the fibers, they are brought into contact with a binding compound. If binding activity occurs, a thermal signal indicative thereof will be transmitted through the fiber to sensor **108**. Since ambient conditions are identical for all fibers, even the slightest fiber-to-fiber variations in activity will be noticeable. In some embodiments, unknown chemical entities can be identified by analysis of the thermal signals.

Brief Description of the Drawings

[0007] **Figure 1** depicts apparatus **100** in accordance with the illustrative embodiment of the present invention.

[0008] **Figure 2** depicts a physical adaptation at second end **104** of a fiber **102**.

[0009] **Figure 3** depicts a removable tip for use at second end **104** of fiber **102**.

[0010] **Figure 4** depicts apparatus **100** of Figure 1 in use with a sample carrier having more wells.

[0011] **Figure 5** depicts the insertion of the second end **104** of fibers **102** into a well that contains binding compound.

[0012] **Figure 6** depicts a variation of the arrangement shown in Figure 5, wherein second end **104** of fibers **102** are interfaced with a surface that has binding compound immobilized thereon.

Detailed Description

[0013] The inventor recognized that decoding of proteins through various interactions (*e.g.*, protein/protein, protein/small molecule-drug, protein/antibody, protein/peptide, *etc.*) by monitoring slight energy-level variations during binding is feasible using a ratiometric (comparative) evaluation of these events through thermal balance (*i.e.*, infrared-radiation level).

[0014] It is very difficult to measure absolute changes in thermal-energy variations during binding. Doing so requires special calorimetric chambers. But it is possible to monitor very small differences in activity between reference "blank" sites and other unknown sites that are being subjected to investigation. This ratiometric approach is

valid only if substantially all other parameters that affect the thermal balance are constant and if the events under investigation are taking place simultaneously.

[0015] The inventive device provides an opportunity for massively-parallel investigation of binding activities by monitoring thermal emission from hundreds or thousand of separate sites.

[0016] Figure 1 depicts apparatus **100** for investigating binding activity in accordance with the illustrative embodiment. As depicted in Figure 1, apparatus **100** includes a plurality of infrared-radiation ("IR")-transmitting fibers **102**, sensor(s) **108**, and sliding separator **110**.

[0017] A variety of IR-transmitting fibers are commercially available, such as chalcogenide glass, polycrystalline IR ("PIR") fibers, and heavy metal fluoride glass ("HMFG") and suitable for use in conjunction with the illustrative embodiment of the present invention.

[0018] First end **104** of each fiber **102** is advantageously optically coupled to a dedicated sensing element (*e.g.*, a pixel, *etc.*) or dedicated group of sensing elements of sensor **108**. Furthermore, fibers **102** are bound or otherwise immobilized near first end **104**. In some embodiments, first end **104** of each of fibers **102** is optically coupled and physically attached (*e.g.*, via index-matching epoxy, *etc.*) to sensor **108**. In some other embodiments, fibers **102** are immobilized near first end **104** by a suitable clamp (not depicted), *etc.*, rather than being attached directly to sensor **108**. But regardless of whether or not fibers **102** are attached to sensor **108**, they are optically coupled to it.

[0019] In some embodiments, sensor **108** is a thermal-sensing element, as is known in the art. For example, the sensor can be a mercury-cadmium-telluride-based device. In some other embodiments, sensor **108** is a MOS, Bolometric, or other type of thermally-sensitive array.

[0020] To capture a chemical entity for study, second end **106** of fibers **102** is "dipped" into sample carrier **112**. The dipping process can be manual or automated in known fashion. The wells in sample carrier **112** contain, in various embodiments, protein or other small molecules. Different entities, known or unknown can be present in various wells. Also, for ratiometric studies, every other well can be empty or can contain a known material for reference.

[0021] In some embodiments, the ability of second end **106** of fiber **102** to capture and immobilize a chemical entity is enhanced by one or more physical adaptations. In some embodiments, the physical adaptation is the shape of second end **106** of fiber **102**, such as a concave shape, as is depicted in FIG. 2. This results in the end of the fiber retaining a predetermined amount of liquid and chemical entity. In some other embodiments, fiber **102** is equipped with a capillary intake whereby capillary action is used to retain a chemical entity. In yet some additional embodiments, the second end **106** of fiber **102** is modified by plasma treatments to render it hydrophilic or hydrophobic, as desired. These physical adaptations promote capture and immobilization of a chemical entity.

[0022] As depicted in Figure 3, in some embodiments, removable tip **314** is added to second end **104** so that the removable tip, rather than the end of the fiber is contaminated by material in the wells of sample carrier **112**. In some embodiments, removable tip **314** is physically adapted to promote capture and immobilization of a chemical entity as described above.

[0023] Sliding separator **110** fans out fibers **102** in a predetermined manner and pattern to fit sample carrier **112** (*e.g.*, micro-titer plates having 96, 384, or 1536 wells, *etc.*) of a variety of configurations. In particular, the position of sliding separator **110** along fibers **102** determines the distance between second ends **106** of adjacent fibers **102**. Assume, for example, sample carrier **112** of Figure 1 is a 96-well micro-well plate and sample carrier **112** of Figure 4 is a 384-well plate. The wells in a 96-well plate are spaced further apart than the wells in a 384 well plate. As a consequence, second ends **106** of adjacent fibers **102** must be further apart when used in conjunction with a 96-well plate than when used with a 384-well plate. This is accomplished by simply positioning sliding separator **110** relatively further from second end **106** of fibers **102** for use with a 96-well plate and relatively closer to second end **106** of fibers **102** for use with a 384-well plate.

[0024] Once the chemical entities are engaged to second end **106** of fibers **102**, the fibers are, in some embodiments, simultaneously inserted into well **516**, as depicted in FIG. 5. Simultaneous insertion is particularly desirable, if not necessary, for ratiometric studies. In some embodiments, well **516** contains a binding compound. Based on knowledge of the behavior of the binding compound with various compounds, the unknown entities obtained from sample carrier **112** can be

identified. The binding compound can be a small molecule drug, an antibody, a protein, a peptide, *etc*.

[0025] To facilitate simultaneous insertion into well **516**, apparatus **100** includes collar **518** for bundling fibers **102**. In some embodiments, collar **518** is slid over fibers **102** at second end **106** after the fibers have received the chemical entities during the first "dipping" operation.

[0026] If binding activity (*i.e.*, between a chemical entity from sample carrier **112** and the binding compound) occurs at second end **106** of any individual fiber **102**, a thermal signal indicative thereof will be transmitted to sensor **108**. Since ambient conditions are identical for all fibers, even the slightest fiber-to-fiber variations in activity will be noticeable.

[0027] As depicted in Figure 6, in some embodiments, rather than dipping fibers **102** in well **516** to receive binding compound, the fibers, with chemical entity **618** attached at second end **106**, are interfaced with binding chemical **620** that is immobilized or otherwise present on surface **622**. The surface is advantageously IR reflective and is advantageously shaped (*e.g.*, flat, parabolic, *etc.*) to direct maximum signal strength toward sensor **108**.

[0028] It is to be understood that the above-described embodiments are merely illustrative of the present invention and that many variations of the above-described embodiments can be devised by those skilled in the art without departing from the scope of the invention. For example, in this specification, specific details are provided in order provide a thorough description and understanding of the illustrative embodiments of the present invention. Those skilled in the art will recognize, however, that the invention can be practiced without one or more of those details, or with other methods, materials, components, *etc*.

[0029] Furthermore, in some instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the illustrative embodiments. It is understood that the various embodiments shown in the Figures are illustrative, and are not necessarily drawn to scale. Furthermore, the particular features, structures, materials, or characteristics can be combined in any suitable manner in one or more embodiments. It is therefore intended that such variations be included within the scope of the following claims and their equivalents.